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(54) **Aspartic proteinase 2 (ASP2)**

(57) ASP2 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing ASP2 polypeptides and polynucle-

otides in the design of protocols for the treatment of Alzheimer's Disease, cancer, and prohormone processing, among others, and diagnostic assays for such conditions.

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**Description**

This application claims the benefit of U.K. Application No. 9701684.4, filed January 28, 1997, which is herein incorporated by reference in its entirety.

**FIELD OF INVENTION**

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Aspartic Proteinase family, hereinafter referred to as ASP2. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

**BACKGROUND OF THE INVENTION**

There are currently five known human aspartic proteases, namely, pepsin, gastricsin, cathepsin D, cathepsin E and renin, and these have widely varying functions. Pepsin and gastricsin are involved in nutritive processes in the stomach, cathepsin D is involved in protein turnover in many cell types, and renin has the highly specific function of angiotensin production from its precursor form, angiotensinogen. The precise role of cathepsin E remains to be confirmed, although its location in some epithelial cells types has indicated a role in antigen processing. It may also be involved in certain inflammatory conditions, such as *Helicobacter pylori* infection in the stomach. This indicates that the Aspartic Proteinase family has an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of Aspartic Proteinase family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, Alzheimer's Disease, cancer, and prohormone processing.

**SUMMARY OF THE INVENTION**

In one aspect, the invention relates to ASP2 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such ASP2 polypeptides and polynucleotides. Such uses include the treatment of Alzheimer's Disease, cancer, and prohormone processing, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with ASP2 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate ASP2 activity or levels.

**DESCRIPTION OF THE INVENTION****Definitions**

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"ASP2" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO: 2 or an allelic variant thereof.

"ASP2 activity or ASP2 polypeptide activity" or "biological activity of the ASP2 or ASP2 polypeptide" refers to the metabolic or physiologic function of said ASP2 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said ASP2.

"ASP2 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide"

refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NYAcad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity, as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity and "similarity can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990). The BLAST X program is publicly available from NCBI

and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894; Altschul, S., *et al.*, *J. Mol. Biol.* 215: 403-410 (1990)). The well known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following:

1) Algorithm: Needleman and Wunsch, *J. Mol. Biol.* 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA.*

89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, *J. Mol. Biol.* 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polynucleotide comparisons.

Preferred polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to a polynucleotide reference sequence of SEQ ID NO:1, wherein said reference sequence may be identical to the sequence of SEQ ID NO: 1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO: 1 by the numerical percent of the respective percent identity and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

wherein  $n_n$  is the number of nucleotide alterations,  $x_n$  is the total number of nucleotides in SEQ ID NO:1, and  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Preferred polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said reference sequence may be identical to the sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity and subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y),$$

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2, and  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85 %, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

## Polypeptides of the Invention

In one aspect, the present invention relates to ASP2 polypeptides (or ASP2 proteins). The ASP2 polypeptides include the polypeptide of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO: 2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within ASP2 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Preferably ASP2 polypeptide exhibit at least one biological activity of ASP2.

The ASP2 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the ASP2 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned ASP2 polypeptides. As with ASP2 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of ASP2 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of ASP2 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate ASP2 activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the ASP2, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The ASP2 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

## Polynucleotides of the Invention

Another aspect of the invention relates to ASP2 polynucleotides. ASP2 polynucleotides include isolated polynucleotides which encode the ASP2 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, ASP2 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a ASP2 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS: 1 and 3. ASP2 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the ASP2 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under ASP2 polynucle-

otides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such ASP2 polynucleotides.

ASP2 of the invention is structurally related to other proteins of the Aspartic Proteinase family, as shown by the results of sequencing the cDNA encoding human ASP2. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 1 to 1503) encoding a polypeptide of 501 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 48.7% identity (using FASTA (GCG)) in 460 amino acid residues with ASP1, Novel Aspartic Proteinase, (U.S. Serial No. Unassigned, Attorney Docket Number GH70262, filed October 6, 1997). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 59.2% identity (using FASTA (GCG)) in 1516 nucleotide residues with ASP1 Novel Aspartic Proteinase (U.S. Serial No. Unassigned, Attorney Docket number GH70262, filed October 6, 1997). Thus, ASP2 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

**Table 1<sup>a</sup>**

```

ATGG CCAAG CCCTGCCCTGG CT CCTGCTGTGGATGGG OG GGGAGTGTGCTG CCTGCCCAO GGCACCCAG
CAO GGCAT COGGCTG CCCCTG OG CAG OGG CCTGGGGGG OG CCCCCCTGGGG CTG OGGCTG CCCOGGGAG
ACOGA OGAAGAG CCOGAGGAG CCOGG COGGAGGGG CAG CTTTGTGGAGATGGTGGACAACCTGAGGGG C
AAGT OGGGG CAGGG CTACT ACGTGGAGATGACOGTGGG CAG CCCCCCGCAGAOCT CAACAT CCTGGTG
GATACAGG CAGCAGTAACTTTG CAGTGGGTGCTG CCCCCCA CCCCTT CCTG CAT OGCTACT ACCAGAGG
CAGCTGT CCAGCACAT ACOGGGACCT COGGAAGGTGTGT ATGAGCCCTACACCCAGGG CAAGTGGGAA
GGGGAGCTGGG CACOGACCTGGT AAGCAT CCCCCATGG CCCCACGT CACTGTG OGTGCCAACATTGCT
GCCAT CACTGAAT CAGACAAGTT CTT CAT CAACGGCT CCAACTGGGAAGGCAT CCTGGGGCTGG CCTAT
GCTGAGATTGCCAGGCCTGAO GACT CCCTGGAGCCTTT CTTTGACT CTCTGGTAAAG CAGACCCAGTT
CCCAACCT CTT CT CCCTG CAGCTTTGTGGTGTGGCTT CCCCCTCAACCAGT CTGAAGTGTGG CCTCT
GT OGGAGGGAGCATGAT CATTGGAGGTAT OGACCACT OGCTGTACACAGG CAGT CTCTGGTATACACCC
ATCOGGOGGGAGTGGTATATGAGGTGAT CATTGTG OGGGTGGAGAT CAATGGACAGGAT CTGAAAATG
GACTG CAAGGAGTACAACATATGACAAGAGCATTGTGGACAGTGGCACCACCAACCTT OGTTTGCCAAG
AAAGTGTGTTGAAGCTG CAGTCAAATCCAT CAAGGCAGCCT CCTCCAOGGAGAAGTT CCCTGATGGTTT C
TGGCTAGGAGAGCAGCTGGTGTGCTGGCAAGCAGGCACCA CCCCCTTGGAA CATTTT CCCAGT CATCTCA
CTCTACCTAATGGGTGAGGTTACCAACCACT CCTTCOGCAT CACCAT CCTTCOGCAGCAATACCTG OGG
CCAGTGGAAGATGTGGCCAOGT CCAAGAOGACTGTTACAAGTTTGCCATCTCACAGT CATCCAOGGG C
ACTGTTATGGGAGCTGTTATCATGGAGGGCTTCTAOGTTGTCTTTGAT OGGGCCOGAAAAO GAATTGGC
TTTGCTGT CAGOGCTTGCCATGTGCAOGATGAGTT CAGGA OGGCAG OGGTGGAAGGCCCTTTTGT CACC
TTGGACATGGAAGACTGTGGCTACAACATT CCACAGACAGATGAGTCAACCT CATGACCATAGCCTAT
GT CATGGCTGCCATCTG OGCCCTCTT CATGCTGCCACTCTGCCTCATGGTGTGT CAGTGGOGCTGCCTC
OGCTGCCTG OGCCAGCAGCATGATGACTTTGCTGATGACATCTCCCTGCTGAAGTGAGGAGGCCCATGG
GAGAAAGATAGAGATTCCCCTGGGACCACACCTCOGTGGTTCACTTTGGT CACAAGTAGGAGACACAGA
TGGCACCTGTGGCCAGAGCACCTCAGGACCCTCCCCACCCACCAATGCCTCTGCCTTGATGGAGAAGG
AAAAGGCTGGCAAGGTGGGTTCCAGGGACTGTACCTGTAGGAAA CAGAAAAGAGAAGAAAGAAGCACTC
TGCTGGCGGGAATACTCTTGGT CACCTCAAATTTAAGT OGGGAAATCTGCTGCTTGAACTT CAGCCC
TGAACCTTTGTCCACCATTCCTTTAAATTCTCCAACCCAAAGTATCTTCTTTTCTTAGTTT CAGAAGT
ACTGGCATCACACGCAGGTTACCTTGGOGTGTGTCCCTGTGGTACCOGGGCAGAGAAGAGACCAAGCTT
GTTTCCCTGCTGGCCAAAGT CAGTAGGAGAGGATGCACAGTTTGCTATTTGCTTTAGAGACAGGGACT

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GT AT AAA CAAG CCT AAC ATT GGT G CAA AG AT T G CCT CTT GA AT T AAAAAAAAAA CT AG AT T GACT AT T  
 T AT ACAA AT GGGG GGG CT GGA AG AGG AGA AGG AG AGG GAG T A CAA AGA CAG GGA AT AG T GGG AT CAA  
 AG CT AGG AAA AGG CAG AAA CACA CCA CT CAC CAG T CCT AG TTT T AGA CCT CAT CT CCA AG AT AG CAT CC  
 CAT CT CAGA AG AT GGG T GT TTT T CAAT GT TTT T CTT T CT GT GGT T G CAG CCT GAC CAAA AG T GAG AT  
 GGG AAG GGT CT AT CT AG CCA AG AG CT CTT TTT T AG CT CT CTT AA AT GA AG T G CCA CT AAG GA AG T T C  
 CACT TGA ACAT GGA AT T T CT GCC AT ATT AAT T T CC AT T GT CT CT AT CT GGA ACC ACC CTT T AAT CT C  
 TAC AT AT GAT T AGG T CCAG CACT T GAAA AT ATT CCT AAC CNA AT T T GNCT TGGGG G CTT T G CNG N  
 CCAG GT G CT AAA AGG G NT TGGG T AGG NG NCC NCT T NT AT NT NAT NCCT NAAA AGGT T ANNG

<sup>a</sup> A nucleotide sequence of a human ASP2 (SEQ ID NO: 1).

**Table 2<sup>b</sup>**

MAQALPWLLLWMGAGVLP AHGTQHGI RLPL RSLGGLAP LGL RLP RETDEEPEEPG RRG SFVEMVD NL RG  
 K SGQGYVEMTVG SPPQTLN ILVDTG SSNFAVGAAPHP FLH RYQ RQL SST Y RDL RKG VYEPYT QGKWE  
 GELGTDLV SIPHGP NVT V RANIAAITE SDKFFI NG SNWEGILGLAYAE IARPDD SLEP FFD SLVKQTHV  
 PNL FSLQL CGAGFPLNQ SEVLASVGG SMI IGGIDH SLYT GSLWYTP I RREWYEV IIV RVEINGQDLKM  
 DCKEYNYDK SIVD SGT TNL RLPKKVFEEAVK SIKAA SSTEKFPD GFWLGEQLV CWQAGTTPWNI FPV I S  
 LYL MGEVT NQ SF RIT I LPQQYL RPVEDVAT SQDD CYKFAI SQ SSTGT VMGAVI MEGFYVVF D RAR RIG  
 FAV SACHVHDEF RTAAVEGP FVT LD MED CGYNIPQTDE STLMT IAYVMAAI CALFMLPL CLMV CQWRCL  
 RCL RQQHDD FADD I SLLK

<sup>b</sup> An amino acid sequence of a human ASP2 (SEQ ID NO: 2).

One polynucleotide of the present invention encoding ASP2 may be obtained using standard cloning and screening,  
 from a cDNA library derived from mRNA in cells of human pancreas and brain, using the expressed sequence tag  
 (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) 355:632-634;  
 Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural  
 sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding ASP2 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding  
 sequence contained in Table 1 (nucleotide number 1 to 1503 of SEQ ID NO:1), or it may be a sequence, which as a  
 result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of ASP2 polypeptide, the poly-  
 nucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding  
 sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding  
 a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For  
 example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred  
 embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE  
 vector (Qiagen, Inc.) and described in Gentz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The  
 polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences,  
 splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding ASP2 variants comprising the amino acid sequence  
 of ASP2 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are  
 substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is  
 contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

**Table 3<sup>f</sup>**

5 GG CAG CTTT GT GGAGAT GGT GGACAACCT GAGGGG CAAGT OGGGG CAGGG CT ACT AOGT GGAGAT GACC  
 GT GGG CAG CCCCCO GAGA OG CT CAACAT CCT GGT GGAT ACAGG CAG CAGT AACTTT G CAGT GGGT G CT  
 G CCCCCA CCCCCTT CCT G CAT OG CT ACT ACCAGAGG CAG CT GT CCAG CACAT ACOGGGACCT COGGAAG  
 GGT GT GT AT GAG CCCT ACACCCAGGG CAAGT GGG AAGGGGAG CT GGG CACOGACCT GGT AAG CAT CCCC  
 10 CAT GG CCCC AAOGT CACT GT G CGT G CCAACATT G CT G CCAT CACT GAAT CAGACAAGTT CTT CAT CAAC  
 GG CT CCAACT GGAAGG CAT CCT GGGG CT GG CCT AT G CT GAGATT G CCAGG CCT GAOGACT CCCT GGAG  
 CCTTT CTTTGACT CT CT GGT AAAG CAGACCCAOGTT CCCAACCT CTT CT CCCT G CAG CTTT GT GGT G CT  
 15 GG CTT CCCCCT CAACCACT CT GAAGT G CT GG CCT CT GT OGGAGGGAG CAT GAT CATTGGAGGT AT OGAC  
 CACT OG CT GT ACACAGG CAGT CT CT GGT AT ACACCCAT COGG OGGGAGT GGT ATT AT GAGGT GAT CATT  
 GT G OGGT GGAGAT CAAT GGACAGGAT CT GAAAT G GACT G CAAGGAGT ACAA CT AT GACAAGAG CATT  
 20 GT GGACAGT GG CACCACCAACCTT OGTTT GCCAAGAAAGT GTTT GAAG CT G CAGT CAAAT CCAT CAAG  
 G CAG CCT CT CCAOGGAGAAGTT CCCT GAT GGT TTT CT GG CT AGGAGAG CAG CT GGT GT G CT GG CAAG CA  
 GG CACCACCCCTT GGAACAT TTT CCCAGT CAT CT CACT CT ACCT AAT GGGT GAGGTT ACCAACCACT CC  
 TT COG CAT CACCAT CCTT COG CAG CAAT ACCT G OGG CCAGT GGAAGAT GT GG CCAOGT CCCAAGACGAC  
 25 TGTT ACAAGTTT G CCAT CT CACAGT CAT CCAOGGG CACT GTT AT GGGAG CT GTT AT CATGGAGGG CTT C  
 T AOGTT GT CTTT GAT OGGG CCOGAAAACGAATT GG CTTT G CT GT CAG OG CTT G CCAT GT G CACGAT GAG  
 TT CAGGAOGG CAG CGT GGAAGG CCCTTTT GT CACCTT GGACAT GGAAGACT GT GG CT ACAACATT CCA  
 30 CAGACAGAT GAGT CAACCCT CAT GACCAT AG CCT AT GT CATGG CT GCCAT CT G OG CCCT CTT CAT G CT G  
 CCACT CT G CCT CAT GGT GT GT CAGT GG OG CT GCCT COG CT G CCT G OG CCAGACAAT GGAT GACTTT G CT  
 GAT GACAT CT CCCT G CT GAAGT GAGGAGG CCCAT GGGAGAAAGAT AGAGATT CCCCTGGGACCACACCT  
 35 COGT GGT T CACTTT GGT CACAAGT AGGAGACACAGAT GG CACCT GT GG CCAGAG CACCT CAGGACCCCT C  
 CCCACCCACCAAAT GCCT CT G CCTT GAT GGAGAAGGAAAAGG CT GG CAAGGT GGGTT CCAGGGACT GT A



CCTGTAGGAAACAGAAAAGAGAAGAAAGAAGCACTCTGCTGGCGGAATACTCTTGGTCACTCAAATT  
 TAAGTGGGAAATTCTGCTGCTTGAAACCTCAGCCCTGAACCTTTGTCCACCATTCTTTAAATTCTCC  
 5 AACCCTAAGTATTCTTCTTTCTTAGTTTCAGAAGTACTGGCATCACACGAGGTTACCTTGGCGTGTG  
 TCCCTGTGGTACCGGGCAGAGAAGAGACCAAGCTGTTTCCCTGCTGGCCAAAGTCAGTAGGAGAGGA  
 TGCAAGTTTGCTATTTGCTTAGAGACAGGGACTGTATAACAAGCCTAACATTGGTGCAAAGATTGC  
 10 CTCTTGAATTAAAAAAAAAACTAGATTGACTATTTATACAAATGGGGGCGGCTGGAAAGAGGAGAAGG  
 AGAGGGAGTACAAAGACAGGGAATAGTGGGATCAAAGCTAGGAAAGGCAGAAACACAACCACTCACCAG  
 TCTAGTTTTAGACCTCATCTCCAAGATAGCATCCCATCTCAGAAGATGGGTGTTGTTTTCAATGTTTT  
 15 CTTTTCTGTGGTTCAGCCTGACCAAAAGTGAGATGGGAAGGCTTATCTAGCCAAAGAGCTCTTTTTT  
 AGCTCTCTTAATGAAGTGCCCACTAAGGAAGTTCACTTGAACACATGGAATTTCTGCCATATTAAAT  
 TCCATTGTCTCTATCTGGAAACCACTTTAATCTCTACATATGATTAGGTCCAGCACTTGAAAATATTC  
 20 CTAAACCNAATTTGCTTGGGGGCTTTCNGNCCAGGTGCTAAAAGGNTTGGGTAGGNGNCCNCTTNT  
 ATNTNATNCCTNAAAAGGTTANNG

A partial nucleotide sequence of a human ASP2 (SEQ ID NO: 3).

**Table 4<sup>d</sup>**

GSFVEMVDNLRGKSGQGYVEMTVGSPQTNLNLVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRLRK  
 30 GUYEPTYQGWEGELGTDLVSIHPGNVTVRANIAAITE SDKFFINGSNWEGILGLAYAEIARPD SLE  
 PFFDSL VKQTHVP NLF SLQLCGAGFPLNQSEVLASVGGSMIIGGIDHSLYTGLWYTPIRREWYEVII  
 VRVEINGQDLKMDCKEYNYDKSIVDSGTTNLRPKKVFEAAVKSIKAA SPREKFPDGFWLGEQLVCWQA  
 35 GTTPWNI FPI SLVLMGEVTNQSFRTITLPQQYL RPVEDVAT SQDDCYKFAI SQSSTGTVMGAVIMEGF  
 YVVFDRARKRIGFAVSACHVHDEFRTAAVEGPFVTLDMEDCGYNIPQTDESTLMTIAYVMAAI CALFML  
 PLCLMVQWRCLRCLRQTMDDFADDISLLK. GGPWEKD RSPGTT PPWFTLVT SRRH RWHLP EHLRTL  
 PTHQMP LP. WRRKRLARWVPGTVPVGNRKEKKEALCWREYSWSPQI. VGKFCCLKLP. T FVHH SFKFS  
 40 NPKYSSFLSFRSTGITRRLPWRVSLWYPGREETKLV SLLAKVSRGCTVCYLL. RQGLYQA. HWCKDC  
 LLN. KKKLD. LFIQMGAAGKRRRGSTKTGNSGIKARKGRNTTHQS. F. TSSP RHPISDGCCFQCF  
 LFGCSLTKSEMGRAYLAKELFFSLK. SAH. GSST. THGISAILI SIVSIWNHPLISTYD. VQHLKIF  
 45 LTXIXLGGFAXQVLKGXG. XXXXXXLKLX

A partial amino acid sequence of a human ASP2 (SEQ ID NO: 4).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences.  
 50 In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to  
 the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will  
 occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more  
 preferably 97-99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in  
 55 SEQ ID NO:1 or a fragment thereof (including that of SEQ ID NO:3), may be used as hybridization probes for cDNA  
 and genomic DNA, to isolate full-length cDNAs and genomic clones encoding ASP2 polypeptide and to isolate cDNA  
 and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human)  
 that have a high sequence similarity to the ASP2 gene. Such hybridization techniques are known to those of skill in

the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding ASP2 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO: 3), and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Thus in another aspect, ASP2 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO:3). Also included with ASP2 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

## Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the ASP2 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If ASP2 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

ASP2 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide

is denatured during isolation and or purification.

### Diagnostic Assays

This invention also relates to the use of ASP2 polynucleotides for use as diagnostic reagents. Detection of a mutated form of ASP2 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of ASP2. Individuals carrying mutations in the ASP2 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled ASP2 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotide probes comprising ASP2 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to Alzheimer's Disease, cancer, and prohormone processing through detection of mutation in the ASP2 gene by the methods described.

In addition, Alzheimer's Disease, cancer, and prohormone processing, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of ASP2 polypeptide or ASP2 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an ASP2 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagnostic kit for a disease or susceptibility to a disease, particularly Alzheimer's Disease, cancer, and prohormone processing, which comprises:

- (a) a ASP2 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a ASP2 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or
- (d) an antibody to a ASP2 polypeptide, preferably to the polypeptide of SEQ ID NO: 2. It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

### Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

A chromosomal loci of 11q22 has been inferred for ASP2 by homology (99% in 210 nucleotides) with Genbank Locus G24698 (Human STS WI-14206).

## Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the ASP2 polypeptides. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the ASP2 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against ASP2 polypeptides may also be employed to treat Alzheimer's Disease, cancer, and prohormone processing, among others.

## Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with ASP2 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from Alzheimer's Disease, cancer, and prohormone processing, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering ASP2 polypeptide via a vector directing expression of ASP2 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a ASP2 polypeptide wherein the composition comprises a ASP2 polypeptide or ASP2 gene. The vaccine formulation may further comprise a suitable carrier. Since ASP2 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

## Screening Assays

The ASP2 polypeptide of the present invention may be employed in a screening process for compounds which activate (agonists) or inhibit activation of (antagonists, or otherwise called inhibitors) the ASP2 polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, enzymes, receptors, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

ASP2 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate ASP2 polypeptide on the one hand and which can inhibit the function of ASP2 polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as Alzheimer's Disease, cancer, and prohormone processing. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as Alzheimer's Disease, cancer, and prohormone processing.

In general, such screening procedures may involve using appropriate cells which express the ASP2 polypeptide

or respond to ASP2 polypeptide of the present invention. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells which express the ASP2 polypeptide (or cell membrane containing the expressed polypeptide) or respond to ASP2 polypeptide are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells which were contacted with the candidate compounds is compared with the same cells which were not contacted for ASP2 activity. In addition, all aspartic proteinases are inhibited by pepstatin. Therefore, pepstatin inhibitory assays may also be employed with the present invention as a method of detection or as a screening assay.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the ASP2 polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the ASP2 polypeptide, using detection systems appropriate to the cells bearing the ASP2 polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing a ASP2 polypeptide to form a mixture, measuring ASP2 activity in the mixture, and comparing the ASP2 activity of the mixture to a standard.

The ASP2 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of ASP2 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of ASP2 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of ASP2 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The ASP2 protein may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the ASP2 is labeled with a radioactive isotope (eg 125I), chemically modified (eg biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. In addition to being used for purification and cloning of the receptor, these binding assays can be used to identify agonists and antagonists of ASP2 which compete with the binding of ASP2 to its receptors, if any. Standard methods for conducting screening assays are well understood in the art.

Examples of potential ASP2 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, enzymes, receptors, etc., as the case may be, of the ASP2 polypeptide, e.g., a fragment of the ligands, substrates, enzymes, receptors, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for ASP2 polypeptides; or compounds which decrease or enhance the production of ASP2 polypeptides, which comprises:

- (a) a ASP2 polypeptide, preferably that of SEQ ID NO:2;
- (b) a recombinant cell expressing a ASP2 polypeptide, preferably that of SEQ ID NO:2;
- (c) a cell membrane expressing a ASP2 polypeptide; preferably that of SEQ ID NO: 2; or
- (d) antibody to a ASP2 polypeptide, preferably that of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

#### Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, Alzheimer's Disease, cancer, and pro-hormone processing, related to both an excess of and insufficient amounts of ASP2 polypeptide activity.

If the activity of ASP2 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the ASP2 polypeptide, such as, for example, by blocking the binding of ligands, substrates, enzymes, receptors, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of ASP2 polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous ASP2 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the ASP2 polypeptide.

In another approach, soluble forms of ASP2 polypeptides still capable of binding the ligand in competition with endogenous ASP2 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the ASP2 polypeptide.

In still another approach, expression of the gene encoding endogenous ASP2 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of ASP2 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates ASP2 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of ASP2 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of ASP2 polypeptides in combination with a suitable pharmaceutical carrier.

### Formulation and Administration

Peptides, such as the soluble form of ASP2 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

### Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

#### Cloning:

Rapid amplification of cDNA ends polymerase chain reaction technology (RACE PCR) was used to identify the

missing 5' cDNA sequence of the aspartyl protease 2 gene. The source of cDNA template for the amplification reactions was a range of Marathon-Ready™ cDNA preparations (Clontech Laboratories, Inc., 1020 East Meadow Circle, Palo Alto, CA 94303-4230, USA.). These Marathon-Ready cDNAs are essentially cDNA libraries which have oligonucleotide adaptors ligated onto them. This allows the researcher to perform 5' RACE PCR using two primers, one complementary to a region of known sequence in the gene of interest and the other complementary to the ligated adaptor; resulting in an extension to the known sequence at the 5' end. PCR was performed using AmpliTaq® Gold DNA polymerase (Perkin-Elmer Corp).

It was found to be necessary to include 5% Dimethylsulphoxide in the reaction buffer for successful amplification, probably due to the high GC nucleotide content of this region of DNA.

The DNA sequence was cloned and a region of DNA was confirmed (nucleotides 1-273 in Table 1 ) at the 5' end of the Asp2 gene as extending from the start codon to overlap with the previously identified EST sequences. This novel sequence was identified in cDNA templates from seven human tissues, heart, leukocyte, mammary gland, spleen, skeletal muscle, thymus and aorta.

#### Northern Analysis:

A human Multiple Tissue Northern blot (MTN catalogue number 7760-1) (Clontech) was hybridized with an Asp-2 specific probe (of 325 nucleotides in length) generated by PCR, using the specific oligonucleotides 5' GATGAGT-TCAGGACGGCAG 3' (SEQ ID NO:5) and 5' GGTGCCATATGTGTCTCC 3' (SEQ ID NO:6). The probe was radiolabelled by incorporation of <sup>32</sup>P-dCTP during PCR amplification, and the labelled PCR product was subsequently purified using the Qiagen PCR Purification Kit. After a 1 hour prehybridization, hybridization was carried out for 2 hours using ExpressHyb buffer (Clontech) at 68°C, and the labelled probe was added to a final concentration of 1×10<sup>6</sup> cpm/ml. After hybridization, the membrane was washed twice in 2×SSC/ 0.05% SDS for 20 minutes, and twice in 0.1×SSC/ 0.1% SDS at 50°C for 20 minutes. The membrane was then wrapped in plastic wrap and exposed to X-ray film at -70°C with two intensifying screens. This revealed that the highest expression (tissues examined were heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) of Asp2 was in the pancreas, followed by the brain.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham p.l.c. and  
SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: ASP2

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: F J Cleveland & Company

(B) STREET: 40/43 Chancery Lane

(C) CITY: London

(D) COUNTY:

(E) COUNTRY: United Kingdom

(F) POST CODE: WC2A 1JQ

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: TO BE ASSIGNED

(B) FILING DATE: 20-JAN-1998

(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: UK 9701684.4

(B) FILING DATE: 28-JAN-1997

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: CRUMP, Julian Richard John

(B) GENERAL AUTHORISATION NUMBER: 37129

(C) REFERENCE/DOCKET NUMBER: GH-70368



## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +44 171 405 5875

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(C) TELEX:

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2541 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

25	ATGGCCCAAG CCCTGCCCCTG GTCCTGCTG TGGATGGGCG CGGGAGTGCT GCCTGCCAC	60
	GGCACCCAGC ACGGCATCCG GCTGCCCCTG CGCAGCGGCC TGGGGGGCGC CCCCCTGGGG	120
	CTGCGGCTGC CCCGGGAGAC CGACGAAGAG CCCGAGGAGC CCGGCCGAG GGGCAGCTTT	180
30	GTGGAGATGG TGGACAACCT GAGGGGCAAG TCGGGGCAGG GCTACTACGT GGAGATGACC	240
	GTGGGCAGCC CCCCAGCAGC GCTCAACATC CTGGTGGATA CAGGCAGCAG TAACTTTGCA	300
	GTGGGTGCTG CCCCCACCC CTCCTGTCAT CGTACTACC AGAGGCAGCT GTCCAGCACA	360
	TACCGGGACC TCCGGAAGGG TGTGTATGAG CCCTACACC AGGGCAAGTG GGAAGGGGAG	420
35	CTGGGCACCG ACCTGGTAAG CATCCCCCAT GGCCCCAAGC TCACTGTGCG TGCCAACATT	480
	GCTGCCATCA CTGAATCAGA CAAGTTCTTC ATCAACGGCT CCAACTGGGA AGGCATCCTG	540
	GGGCTGGCCT ATGCTGAGAT TGCCAGGCCT GACGACTCCC TGGAGCCTTT CTTTGACTCT	600
	CTGGTAAAGC AGACCCACGT TCCCAACCTC TTCTCCCTGC AGCTTTGTGG TGCTGGCTTC	660
40	CCCCTCAACC AGTCTGAAGT GCTGGCCTCT GTCGGAGGGA GCATGATCAT TGGAGGTATC	720
	GACCACTCGC TGTACACAGG CAGTCTCTGG TATACACCA TCCGGCGGGA GTGGTATTAT	780
	GAGGTGATCA TTGTGCGGGT GGAGATCAAT GGACAGGATC TGAAAATGGA CTGCAAGGAG	840
	TACAACTATG ACAAGAGCAT TGTGGACAGT GGCACCACCA ACCTTCGTTT GCCCAAGAAA	900
45	GTGTTTGAAG CTGCAGTCAA ATCCATCAAG GCAGCCTCCT CCACGGAGAA GTTCCCTGAT	960
	GGTTTCTGGC TAGGAGAGCA GCTGGTGTGC TGGCAAGCAG GCACCACCCC TTGGAACATT	1020
	TTCCAGTCA TCTCACTCTA CCTAATGGGT GAGGTTACCA ACCAGTCCTT CCGCATCACC	1080
50	ATCCTTCCGC AGCAATACCT GCGGCCAGTG GAAGATGTGG CCACGTCCA AGACGACTGT	1140
	TACAAGTTTG CCATCTCACA GTCATCCACG GGCCTGTTA TGGGAGCTGT TATCATGGAG	1200
	GGCTTCTACG TTGTCTTTGA TCGGGCCCGA AAACGAATTG GCTTTGCTGT CAGCGCTTGC	1260
	CATGTGCACG ATGAGTTCAG GACGGCAGCG GTGGAAGGCC CTTTGTGCAC CTTGGACATG	1320
55	GAAGACTGTG GCTACAACAT TCCACAGACA GATGAGTCAA CCCTCATGAC CATAGCCTAT	1380
	GTCATGGCTG CCATCTGCGC CCTCTTCATG CTGCCACTCT GCCTCATGGT GTGTCACTGG	1440

5 CGCTGCCTCC GCTGCCTGCG CCAGCAGCAT GATGACTTTG CTGATGACAT CTCCCTGCTG 1500  
 AAGTGAGGAG GCCCATGGGA GAAAGATAGA GATTCCCCTG GGACCACACC TCCGTGGTTC 1560  
 ACTTTGGTCA CAAGTAGGAG ACACAGATGG CACCTGTGGC CAGAGCACCT CAGGACCCTC 1620  
 CCCACCCACC AAATGCCTCT GCCTTGATGG AGAAGGAAAA GGCTGGCAAG GTGGGTTCCTA 1680  
 GGGACTGTAC CTGTAGGAAA CAGAAAAGAG AAGAAAGAAG CACTCTGCTG GCGGGAATAC 1740  
 TCTTGGTCAC CTCAAATTTA AGTCGGGAAA TTCTGCTGCT TGAAACTTCA GCCCTGAACC 1800  
 10 TTTGTCCACC ATTCCTTTAA ATTCTCCAAC CCAAAGTATT CTTCTTTTCT TAGTTTCAGA 1860  
 AGTACTGGCA TCACACGCAG GTTACCTTGG CGTGTGTCCC TGTGGTACCC GGCAGAGAA 1920  
 GAGACCAAGC TTGTTTCCCT GCTGGCCAAA GTCAGTAGGA GAGGATGCAC AGTTTGCTAT 1980  
 TTGCTTTAGA GACAGGGACT GTATAAACAA GCCTAACATT GGTGCAAAGA TTGCCTCTTG 2040  
 15 AATTAAAAAA AAAAAGTAGA TTGACTATTT ATACAAATGG GGGCGGCTGG AAAGAGGAGA 2100  
 AGGAGAGGGA GTACAAAGAC AGGGAATAGT GGGATCAAAG CTAGGAAAGG CAGAAACACA 2160  
 ACCACTCACC AGTCCTAGTT TTAGACCTCA TCTCCAAGAT AGCATCCCAT CTCAGAAGAT 2220  
 GGGTGTGTGT TTCAATGTTT TCTTTTCTGT GGTTCAGCC TGACCAAAG TGAGATGGGA 2280  
 20 AGGGCTTATC TAGCCAAAGA GCTCTTTTTT AGCTCTCTTA AATGAAGTGC CCACTAAGGA 2340  
 AGTTCCACTT GAACACATGG AATTTCTGCC ATATTAATTT CCATTGTCTC TATCTGGAAC 2400  
 CACCCTTTAA TCTCTACATA TGATTAGGTC CAGCACTTGA AAATATTCCT AACCNNNAATT 2460  
 25 TGNCTTGGGG GCTTTGCNGN CCAGGTGCTA AAAGGGNTTG GGTAGGNGNC CNCTTNTATN 2520  
 TNATNCCTNA AAAGGTTANN G 2541

## (2) INFORMATION FOR SEQ ID NO:2:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 501 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Gln Ala Leu Pro Trp Leu Leu Leu Trp Met Gly Ala Gly Val  
 1 5 10 15  
 45 Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser  
 20 25 30  
 Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp  
 35 40 45  
 50 Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val  
 50 55 60  
 Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr  
 65 70 75 80  
 55 Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser

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	85	90	95
5	Ser Asn Phe Ala Val Gly Ala Ala	Pro His Pro Phe Leu His Arg Tyr	
	100	105	110
	Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val		
	115	120	125
10	Tyr Glu Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp		
	130	135	140
	Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile		
	145	150	155
15	Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp		
	165	170	175
	Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp		
	180	185	190
20	Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro		
	195	200	205
	Asn Leu Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln		
	210	215	220
25	Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile		
	225	230	235
	Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg		
	245	250	255
30	Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln		
	260	265	270
	Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val		
	275	280	285
35	Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala		
	290	295	300
	Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp		
	305	310	315
40	Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr		
	325	330	335
	Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val		
	340	345	350
45	Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg		
	355	360	365
	Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala		
	370	375	380
50	Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val Ile Met Glu		
	385	390	395
	Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala		
	405	410	415
55	Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala Val Glu		
	420	425	430

Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro  
 435 440 445  
 5 Gln Thr Asp Glu Ser Thr Leu Met Thr Ile Ala Tyr Val Met Ala Ala  
 450 455 460  
 Ile Cys Ala Leu Phe Met Leu Pro Leu Cys Leu Met Val Cys Gln Trp  
 465 470 475 480  
 10 Arg Cys Leu Arg Cys Leu Arg Gln Gln His Asp Asp Phe Ala Asp Asp  
 485 490 495  
 Ile Ser Leu Leu Lys  
 500  
 15

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2370 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 25

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30 GGCAGCTTTG TGGAGATGGT GGACAACCTG AGGGGCAAGT CGGGGCAGGG CTACTACGTG 60  
 GAGATGACCG TGGGCAGCCC CCCGACAGCG CTCAACATCC TGGTGGATAC AGGCAGCAGT 120  
 35 AACTTTGCAG TGGGTGCTGC CCCCCACCCC TTCCTGCATC GCTACTACCA GAGGCAGCTG 180  
 TCCAGCACAT ACCGGGACCT CCGGAAGGGT GTGTATGAGC CCTACACCCA GGGCAAGTGG 240  
 GAAGGGGAGC TGGGCACCGA CCTGGTAAGC ATCCCCCATG GCCCAACCGT CACTGTGCGT 300  
 GCCAACATTG CTGCCATCAC TGAATCAGAC AAGTTCTTCA TCAACGGCTC CAACTGGGAA 360  
 40 GGCATCCTGG GGCCTGGCCTA TGCTGAGATT GCCAGGCCTG ACGACTCCCT GGAGCCTTTC 420  
 TTTGACTCTC TGGTAAAGCA GACCCACGTT CCCAACCTCT TCTCCCTGCA GCTTTGTGGT 480  
 GCTGGCTTCC CCCTCAACCA GTCTGAAGTG CTGGCCTCTG TCGGAGGGAG CATGATCATT 540  
 GGAGGTATCG ACCACTCGCT GTACACAGGC AGTCTCTGGT ATACACCCAT CCGGCGGGAG 600  
 45 TGGTATTATG AGGTGATCAT TGTGCGGGTG GAGATCAATG GACAGGATCT GAAAATGGAC 660  
 TGCAAGGAGT ACAACTATGA CAAGAGCATT GTGGACAGTG GCACCACCAA CCTTCGTTTG 720  
 CCCAAGAAAG TGTTTGAAGC TGCAGTCAAA TCCATCAAGG CAGCCTCTCC ACGGGAGAAG 780  
 TTCCCTGATG GTTCTGCGCT AGGAGAGCAG CTGGTGTGCT GGCAAGCAGG CACCACCCTT 840  
 50 TGGAACATTT TCCCAGTCAT CTCCTCTAC CTAATGGGTG AGGTTACCAA CCAGTCCTTC 900  
 CGCATCACCA TCCTTCCGCA GCAATACCTG CGGCCAGTGG AAGATGTGGC CACGTCCCAA 960  
 GACGACTGTT ACAAGTTTGC CATCTCACAG TCATCCACGG GCACTGTTAT GGGAGCTGTT 1020  
 ATCATGGAGG GCTTCTACGT TGTCTTTGAT CGGGCCCGAA AACGAATTGG CTTTGCTGTC 1080  
 55 AGCGCTTGCC ATGTGCACGA TGAGTTCAGG ACGGCAGCGG TGGAAGGCCC TTTTGTCAAC 1140  
 TTGGACATGG AAGACTGTGG CTACAACATT CCACAGACAG ATGAGTCAAC CCTCATGACC 1200

ATAGCCTATG TCATGGCTGC CATCTGCGCC CTCTTCATGC TGCCACTCTG CCTCATGGTG 1260  
 TGTCAGTGGC GCTGCCTCCG CTGCCTGCGC CAGACAATGG ATGACTTTGC TGATGACATC 1320  
 5 TCCCTGCTGA AGTGAGGAGG CCCATGGGAG AAAGATAGAG ATTCCCCTGG GACCACACCT 1380  
 CCGTGGTTCA CTTTGGTCAC AAGTAGGAGA CACAGATGGC ACCTGTGGCC AGAGCACCTC 1440  
 AGGACCCTCC CCACCCACCA AATGCCTCTG CCTTGATGGA GAAGGAAAAG GCTGGCAAGG 1500  
 TGGGTTCAG GGA CTGTACC TG TAGGAAAC AGAAAAGAGA AGAAAGAAGC ACTCTGCTGG 1560  
 10 CGGAATACT CTGGTCACC TCAAATTTAA GTCGGGAAAT TCTGCTGCTT GAAACTTCAG 1620  
 CCCTGAACCT TTGTCCACCA TTCTTTTAAA TTCTCCAACC CAAAGTATTC TTCTTTTCTT 1680  
 AGTTTCAGAA GTACTGGCAT CACACGCAGG TTACCTTGGC GTGTGTCCCT GTGGTACCCG 1740  
 GGCAGAGAAG AGACCAAGCT TGTTTCCCTG CTGGCCAAAG TCAGTAGGAG AGGATGCACA 1800  
 15 GTTTGCTATT TGCTTTAGAG ACAGGGACTG TATAACAAG CCTAACATTG GTGCAAAGAT 1860  
 TGCTCTTGA ATTAAAAAAA AAAACTAGAT TGACTATTTA TACAAATGGG GCGGGCTGGA 1920  
 AAGAGGAGAA GGAGAGGGAG TACAAAGACA GGAATAGTG GGATCAAAGC TAGGAAAGGC 1980  
 AGAAACACAA CCACTCACCA GTCCTAGTTT TAGACCTCAT CTCCAAGATA GCATCCCATC 2040  
 20 TCAGAAGATG GGTGTTGTTT TCAATGTTTT CTTTTCTGTG GTTGCGCCT GACCAAAAGT 2100  
 GAGATGGGAA GGGCTTATCT AGCCAAAGAG CTCTTTTTTA GCTCTCTTAA ATGAAGTGCC 2160  
 CACTAAGGAA GTTCCACTTG AACACATGGA ATTTCTGCCA TATTAAATTC CATTGTCTCT 2220  
 ATCTGGAACC ACCCTTTAAT CTCTACATAT GATTAGGTCC AGCACTTGAA AATATTCCTA 2280  
 25 ACCNNAATTT GNCCTGGGGG CTTGCGNGNC CAGGTGCTAA AAGGGNTTGG GTAGGNGNCC 2340  
 NCTTNTATNT NATNCCTNAA AAGGTTANNG 2370

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 774 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gly Ser Phe Val Glu Met Val Asp Asn Leu Arg Gly Lys Ser Gly Gln  
 1 5 10 15  
 Gly Tyr Tyr Val Glu Met Thr Val Gly Ser Pro Pro Gln Thr Leu Asn  
 20 25 30  
 Ile Leu Val Asp Thr Gly Ser Ser Asn Phe Ala Val Gly Ala Ala Pro  
 35 40 45  
 His Pro Phe Leu His Arg Tyr Tyr Gln Arg Gln Leu Ser Ser Thr Tyr  
 50 55 60  
 Arg Asp Leu Arg Lys Gly Val Tyr Glu Pro Tyr Thr Gln Gly Lys Trp  
 65 70 75 80

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	Glu Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro His Gly Pro Asn	
	85	90 95
5	Val Thr Val Arg Ala Asn Ile Ala Ala Ile Thr Glu Ser Asp Lys Phe	
	100	105 110
	Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly Leu Ala Tyr Ala	
	115	120 125
10	Glu Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu	
	130	135 140
	Val Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu Gln Leu Cys Gly	
	145	150 155 160
15	Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly	
	165	170 175
	Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu	
	180	185 190
20	Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val	
	195	200 205
	Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr	
	210	215 220
25	Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu	
	225	230 235 240
	Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser	
	245	250 255
30	Pro Arg Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val	
	260	265 270
	Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser	
	275	280 285
35	Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile	
	290	295 300
	Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val Ala Thr Ser Gln	
	305	310 315 320
40	Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val	
	325	330 335
	Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala	
	340	345 350
45	Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His Val His Asp Glu	
	355	360 365
	Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu	
	370	375 380
50	Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser Thr Leu Met Thr	
	385	390 395 400
	Ile Ala Tyr Val Met Ala Ala Ile Cys Ala Leu Phe Met Leu Pro Leu	
	405	410 415
55	Cys Leu Met Val Cys Gln Trp Arg Cys Leu Arg Cys Leu Arg Gln Thr	

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[illegible]

Xaa Leu Lys Arg Leu Xaa

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATGAGTTCA GGACGGCAG

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTGCCATAT GTGTCTCC

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**Claims**

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the ASP2 polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.
2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO:1 encoding the ASP2 polypeptide of SEQ ID NO:2.
3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length.
4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.



5. The polynucleotide of claim 1 which is DNA or RNA.
6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a ASP2 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.
7. A host cell comprising the expression system of claim 6.
8. A process for producing a ASP2 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.
9. A process for producing a cell which produces a ASP2 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a ASP2 polypeptide.
10. A ASP2 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.
11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.
12. An antibody immunospecific for the ASP2 polypeptide of claim 10.
13. A method for the treatment of a subject in need of enhanced activity or expression of ASP2 polypeptide of claim 10 comprising:
  - (a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or
  - (b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the ASP2 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.
14. A method for the treatment of a subject having need to inhibit activity or expression of ASP2 polypeptide of claim 10 comprising:
  - (a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or
  - (b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or
  - (c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate, or receptor.
15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of ASP2 polypeptide of claim 10 in a subject comprising:
  - (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said ASP2 polypeptide in the genome of said subject; and/or
  - (b) analyzing for the presence or amount of the ASP2 polypeptide expression in a sample derived from said subject.
16. A method for identifying compounds which inhibit (antagonize) or agonize the ASP2 polypeptide of claim 10 which comprises:
  - (a) contacting a candidate compound with cells which express the ASP2 polypeptide (or cell membrane expressing ASP2 polypeptide) or respond to ASP2 polypeptide; and
  - (b) observing the binding, or stimulation or inhibition of a functional response; or comparing the ability of the cells (or cell membrane) which were contacted with the candidate compounds with the same cells which were not contacted for ASP2 polypeptide activity.
17. An agonist identified by the method of claim 16.

18. An antagonist identified by the method of claim 16.

19. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a ASP2 polypeptide.

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